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14-3-3 Proteins in Pineal Photoneuroendocrine Transduction: How Many Roles?

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Abstract

Recent studies suggest that a common theme links the diverse elements of pineal photoneuroendocrine transduction – *regulation via binding to 14-3-3 proteins*. The elements include photoreception, neurotransmission, signal transduction and the synthesis of melatonin from tryptophan. We review general aspects of 14-3-3 proteins and their biological function as binding partners, and also focus on their roles in pineal photoneuroendocrine transduction.

The function of the pineal gland as a photoneuroendocrine transducer involves multiple processes, including photoreception, signal transduction and the synthesis of melatonin from tryptophan. In addition, in birds and mammals, adrenergic neurotransmission plays a regulatory role. Recent studies suggest that a common theme links these diverse processes, one that has been generally ignored: regulation via binding to 14-3-3 proteins. We review general aspects of 14-3-3 proteins, their biological role as binding partners and their roles in pineal photoneuroendocrine transduction.

14-3-3 proteins: background and biological role as binding partners

14-3-3 proteins are widely distributed in biology (1–5). They were discovered during an effort to characterize brain proteins, which revealed the presence of a relatively abundant group of acidic proteins. The name 14-3-3 reflects their behaviour in DEAE-cellulose chromatography and starch gel electrophoresis (6).

The 14-3-3 family of proteins is comprised of homologous 30 and 33 kDa isoforms that typically occur in cells as soluble hetero- and homodimers. There are seven known mammalian 14-3-3 isoforms, designated β, ε, γ, η, σ, τ and ζ, according to their elution pattern in reverse phase column chromatography. In addition, the phosphorylated forms of the β and ζ isoforms, have been identified as α and δ isoforms, respectively (1–4). There is little known about what controls the relative abundance of each isoform in a particular cell, whether the abundance of any one isoform can be selectively controlled or if 14-3-3 proteins are constitutively expressed as house-keeping genes.

14-3-3 proteins are most abundant in the cytoplasm, although it is clear they can be associated with proteins lodged in membranes. In addition, they occur in the nucleus, reflecting their role in the shuttling of some transcription factors across the nuclear membrane (1–4).

All actions of 14-3-3 proteins involve binding to target proteins, over 100 of which have been identified; there is little doubt that this number will increase many-fold as a result of the current interest in functional proteomics and protein–protein interactions. Review of the known 14-3-3 binding partners reveals that they have little in common on a functional or structural basis, other than the capacity to bind to 14-3-3 proteins. Of special interest to students of pineal photoneuroendocrine transduction is that the first functionally important role established for any 14-3-3 protein was that of an activator of tryptophan and tyrosine hydroxylases (7). In many cases, binding is controlled by serine/threonine phosphorylation of these target proteins. That is, phosphorylation of the target proteins switches them from nonbinding to high affinity binding partners. Through this phosphorylation-dependent binding mechanism, 14-3-3 proteins are critical elements of signal transduction and are involved in a broad range of functions, including actions on cell cycle, apoptosis, protein trafficking, enzyme activation/protection, mitogenic signal transduction, transmitter release, catecholamine and serotonin synthesis, receptor function, ion channel activity and, of special relevance to pineal function, the conversion of serotonin to melatonin (1–4).

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14-3-3 proteins can be viewed as being similar to other ubiquitous signal transduction proteins, such as G proteins, second messenger-regulated kinases and regulatory element binding proteins, because their function in a specific cell type reflects the unique milieu and pattern of gene expression of that cell.

Knowledge of the biology of 14-3-3 proteins is expanding in many directions, and it is well beyond the scope of this paper to provide a thorough review of the status of the field. The interested reader is encouraged to study one or more of the comprehensive reviews from the laboratories of Alastair Aitkin, Haian Fu and Michael Yaffe (1–4).

The structural basis of 14-3-3 binding

What is the chemical basis of sequence-specific binding, and how do 14-3-3 proteins recognize phosphorylated sequences? The answers to these questions are derived from X-ray crystallography. Initial insight was provided by the structures of uncomplexed 14-3-3 ζ and τ (8, 9), which were found to be essentially identical. The basic monomeric unit is a curved molecule consisting of nine, tightly-packed antiparallel α-helices. The remarkable aspect of the structure, from which function can be imagined from form, is the fashion in which two monomers cooperate in the formation of a bowl-shaped dimer. Each monomer contributes one side of the bowl, and the floor is formed by the dimer interface where three *N*-terminal helices from each monomer come together.

Mapping of the conserved residues of the various 14-3-3 isoforms onto the structures showed that the interior surface of the dimer is lined with invariant amino acids, while the variable residues are located on the outside. This suggested that the common function of 14-3-3 molecules (binding other proteins) was likely to be mediated by residues located on the concave surface of the dimer (the inner surface of the bowl). Another intriguing feature of the bowl interior is that each of the two opposing sides contains an amphipathic groove (i.e. an indentation lined with hydrophobic residues along one rim and positively charged amino acids on the other). These grooves are approximately 25 Å long, and are separated from each other by approximately 30 Å.

Analysis of the structures of 14-3-3 proteins cocrystallized with short phosphorylated peptides revealed that the amphipathic grooves are, in fact, the sites of peptide binding (10–12). The structure of 14-3-3 ζ bound to a peptide containing a phosphorylated sequence from the polyoma middle-T antigen indicated that each groove of the 14-3-3 dimer was occupied by peptide bound in an extended conformation (10). Seven peptide residues, centred around phosphoserine, could be unambiguously located, thereby defining the length of the motif recognized by 14-3-3. This result is in excellent agreement with those from screenings of degenerate peptide libraries which showed that the 14-3-3 : peptide interaction region consists of four residues located *N*-terminal and two residues located *C*-terminal to the phosphorylated serine (pSer) (2, 10–12).

Four residues of 14-3-3 are in direct contact with the phosphoserine group: three basic residues (Arg-56, Arg-127, Lys-49) and a tyrosine (Tyr-128). These interact through hydrogen bonds that converge on the phosphate group of pSer, providing a structural explanation for the selectivity for a phosphorylated residue embedded within the recognition motif (10).

A common feature of the peptides that have been cocrystallized with 14-3-3 (and of the recognition sequence in AANAT, discussed below) is the presence of a proline residue at the +2 position relative to the phosphorylated amino acid. Although a proline is not strictly required at this position, it serves the structural purpose of distorting the extended peptide region such that the path of the protein backbone is directed out from the 14-3-3 central cavity (10).

Although it is clear that, in most cases, the phosphorylated residue is required for binding, this is not the sole determinant of recognition and binding, as indicated by the finding that unphosphorylated peptides bind to the amphipathic groove of 14-3-3 (11, 13, 14). This indicates that some sequence flexibility is permitted in the context of motifs that are recognized by 14-3-3 molecules, and that this type of binding might play a functional role in some cells.

14-3-3 proteins present in the mammalian pineal gland

14-3-3 proteins appeared in the pineal literature because of a serendipitous discovery that 14-3-3 isoforms copurify with arylalkylamine *N*-acetyltransferase (AANAT) (5). This finding was an offshoot of attempts to purify AANAT, which was expected to be a bridge towards cloning. It is striking that the 14-3-3 and AANAT coeluted following three column chromatography steps (cystamine-Sepharose affinity, anion-exchange and size-exclusion) (5), inferring a tight association. Sequencing of the most prominent bands indicated they contained 14-3-3 ε and ζ isoforms (5).

The α, β, δ and ε isoforms are dominant in the pineal gland. 14-3-3 ζ is 40% as abundant, the η-isoform is present at low levels and the γ isoform is undetectable (5). It is notable that, although most mammalian 14-3-3 isoforms are present, the partially purified preparation of AANAT was highly enriched with only two. This suggests that there might be ε/ζ isoform selectivity in the 14-3-3/AANAT interaction within the context of the pinealocyte.

Pineal 14-3-3 proteins exhibit changes in abundance during development. The ε and the 30 kDa isoforms seem to vary in a reciprocal manner with 14-3-3 ε highest early in development and decreasing thereafter, whereas 30 kDa isoforms exhibit the reverse pattern (5). This suggests that the expression of some 14-3-3 isoforms in the pineal is developmentally regulated, which may influence function during development. Preliminary studies have indicated that there are no day/night differences in total 14-3-3 levels in the pineal gland (Roseboom, Weller and Klein, unpublished results); photic regulation has not been investigated, nor has the question of how the abundance of individual isoforms is regulated.

Phylogenetic class-dependent roles of 14-3-3 proteins in pineal photoneuroendocrine transduction

The task of summarizing and predicting the various roles that 14-3-3 proteins play or might play in pineal photoneuroendocrine transduction is made somewhat more complex by differences in the organization of vertebrate melatonin rhythm-generating systems (15). Although all pinealocytes can synthesize melatonin, nonmammalian pineal glands also have the ability to detect light (i.e. photoneuroendocrine transduction takes place within a single cell). In contrast, the mammalian pineal gland is part of the multicomponent photoneuroendocrine system that includes the retina and suprachiasmatic nucleus, and neural structures which link them. Accordingly, the functions of specific 14-3-3 proteins and

where they act in the photoneuroendocrine transduction process will vary somewhat from one class to another.

14-3-3 proteins and photoreception

Light regulates pineal function either through effects mediated by the retina, as is the case in mammals and birds, or through direct effects mediated by photodetection systems in pinealocytes, as in birds and fish. In both tissues, this involves similar mechanisms, in which light acts through photopigments to activate G-protein mediated signal transduction.

What role do 14-3-3 proteins play in this process? Recent developments have revealed that 14-3-3 proteins bind to phosducin (Phd, MEKA) (16), a 33-kDa cytosolic protein that is highly expressed in the retina and pineal gland. Phd has the notable ability to bind G protein $\beta\gamma$-heterodimers ($G_{\beta\gamma}$) with high affinity. When bound to Phd, $G_{\beta\gamma}$ is sterically blocked from interacting with the α-subunit of transducin (T_α) or other G_α subunits. Through this mechanism, Phd is thought to down-regulate G protein signal transduction as indicated in Fig. 1 (where 'Light' represents light during the day and 'Dark' represents darkness at night).

Phosphorylation of Phd (pPhd) at Ser-54 and Ser-73 by Ca^{2+}/calmodulin-dependent kinase II (CaMKII) results in binding to 14-3-3 proteins (17, 18). Accordingly, 14-3-3 competes with $G_{\beta\gamma}$ for Phd as a binding partner. Binding of 14-3-3 to pPhd occurs in the dark and appears to 'neutralize' Phd by preventing it from binding to $G_{\beta\gamma}$, thereby enhancing T_α-mediated photodetection. During the day, unphosphorylated Phd would not bind to 14-3-3, but would bind to $G_{\beta\gamma}$, with the resulting down-regulation. In addition to playing a role in photodetection, binding of pPhd to 14-3-3 may also protect against degradation or aggregation (17, 19). It is interesting to note that an argument has also been made for the existence of a 14-3-3-mediated link in the synaptic region of photoreceptors, in which 14-3-3 can either bind to Phd or modulate release of glutamate (18), through a series of events described below for catecholamine release.

The 14-3-3/pPhd mechanism of regulating photosensitivity has been studied in the mammalian retina and is likely to occur in the retinae of other vertebrates. It is also probable that it will function in nonmammalian pineal photoreception. This remains to be established experimentally and the issue is of interest because it is possible that pPhd might compete with other 14-3-3 binding partners in the pinealocyte, an interplay through which 14-3-3 binding partners might influence each other if partners compete for the same binding sites.

14-3-3 proteins and catecholamine biology

Both the synthesis and release of transmitters involved in the transsynaptic regulation of the rat and chicken pinealocyte probably

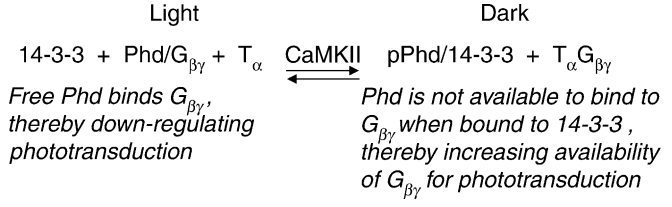

FIG. 1. Regulation of phosducin (Phd) in the mammalian retina.

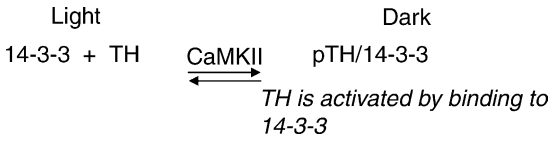

FIG. 2. Hypothetical regulation of tyrosine hydroxylase (TH) in sympathetic terminals in the mammalian pineal gland.

involve 14-3-3 proteins, according to studies with other systems (20–27). The first and rate-limiting step in the synthesis of norepinephrine from tyrosine is hydroxylation by tyrosine hydroxylase (TH, tyrosine 3-monooxygenase, EC 1.14.16.2) (Fig. 2). Several Ser/Thr phosphorylation sites on TH are involved in regulation of enzyme activity. Phosphorylation at Ser-40 by cyclic AMP dependent protein kinase (PKA) or Ca^{2+}/phospholipid dependent protein kinase (PKC) leads to a direct activation of the enzyme by reducing its K_m for the cofactor 6-methyltetrahydropterin ($6MPH_4$) and increasing its K_i for inhibitory catecholamines (20–22). This occurs without the involvement of 14-3-3 proteins. However, phosphorylation at Ser-19 by CaMKII leads to formation of a complex with 14-3-3 protein, resulting in an increase in V_{max} (23–27).

The structural basis of TH/14-3-3 interaction remains unclear because the primary sequence surrounding the Ser-19 does not correspond to the ideal consensus sequence for 14-3-3 binding. It is possible that Ser-40 phosphorylation also plays a role in complex formation (28).

The TH/14-3-3 activation mechanism described here was established using brain preparations. It remains to be determined whether this occurs in the sympathetic nerves terminating in the pineal gland. If this mode of TH regulation exists in avian species, it is expected to be 180° out of phase with the mammalian pattern, because norepinephrine release is increased during the day, when it acts to suppress cAMP production via an action involving α_2-adrenergic receptors (15).

The process of catecholamine release from sympathetic nerve terminals may also involve 14-3-3 proteins, based on studies with permeabilized adrenal chromaffin (PAC) cells. This preparation serves as a model of Ca^{2+}-dependent catecholamine release from sympathetic nerve terminals, such as those innervating the pineal gland (29). Using this model, it has been found that Ca^{2+}-stimulated exocytosis can be enhanced using extracts of brain homogenates; one of the active components, Exo1, was ultimately identified as a 14-3-3 protein.

Exocytosis of chromaffin granules takes place in two steps, priming and triggering (30). The priming step involves ATP-dependent recruitment and docking of vesicles to the cell membrane, whereas triggering is the ATP-independent fusion of the vesicle with the membrane and release of its contents. 14-3-3 functions in the priming phase through two, perhaps related, mechanisms. First, 14-3-3 proteins interact with, and regulate, kinases, including PKC (31). Activation of PKC increases Ca^{2+}-stimulated exocytosis from PAC cells (32) and potentiates the ability of 14-3-3 to reconstitute exocytosis (29). This suggests that 14-3-3 is involved in the effects of PKC on exocytosis.

In addition, 14-3-3 proteins may directly interact with the actin cytoskeleton, which destabilizes the cortical actin network in the PAC cells (33). This filamentous actin network functions as a

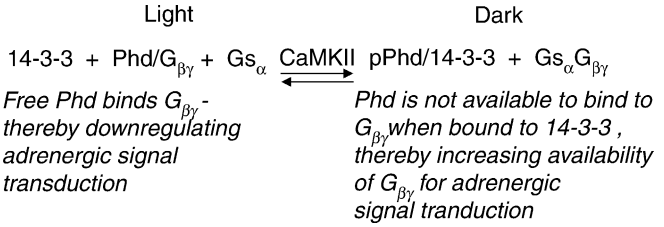

FIG. 3. Hypothetical control of adrenergic sensitivity by phosducin (Phd) in the mammalian pinealocyte.

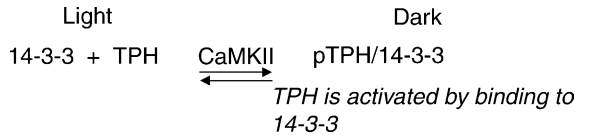

FIG. 4. Hypothetical regulation of tryptophan hydroxylase (TPH) in pinealocytes.

barrier to reduce access of the vesicles to the cell membrane (34). Thus, disrupting this network increases the rate at which vesicles can dock and fuse with the membrane. PKC itself can also promote actin disassembly (35, 36). Accordingly, 14-3-3 and PKC act synergistically to facilitate the priming phase of vesicle release by disrupting the actin network, but the extent to which they interact with each other in this capacity is not clear.

It will be of importance to establish whether 14-3-3 proteins are involved in exocytosis from sympathetic nerves in the pineal gland, as is seen with PAC cells.

14-3-3 proteins and melatonin synthesis

Adrenergic signal transduction

In mammals, melatonin synthesis is turned on by the release of norepinephrine from nerve terminals in the pineal gland, which leads to activation of adrenergic receptors and elevation of cAMP and Ca^{2+}. As in the retina, where Phd regulates photic sensitivity, it is hypothetically possible that a similar mechanism functions in the pinealocyte to control adrenergic receptor sensitivity because the pinealocyte contains high concentrations of Phd (37, 38). When $G_{\beta\gamma}$ is bound to Phd, it seems likely that the system will be down-regulated; phosphorylation of Phd following adrenergic stimulation could regulate this and enhance adrenergic responsiveness, as suggested in Fig. 3.

Tryptophan hydroxylase

Tryptophan hydroxylase (TPH; tryptophan 5-monooxygenase; EC 1.14.16.4) converts L-tryptophan to 5-hydroxy-L-tryptophan, the initial step in the biosynthesis of serotonin and melatonin in the pineal gland from tryptophan. The activity of TPH is influenced by several regulatory mechanisms, including gene expression and cofactor biosynthesis. However, phosphorylation of the enzyme is believed to be of functional significance in mediating changes in activity involving signal transduction (39–41). TPH is activated following phosphorylation by CaMKII (23) and binding to 14-3-3 (7, 42, 43). The binding of 14-3-3 to phosphorylated TPH appears to have two effects: activation resulting in an increased V_{max}; and, stabilization by preventing its dephosphorylation (44, 45) (Fig. 4).

TPH activity has been reported to exhibit a diurnal rhythm with a peak at night in the rat pineal gland, chicken retina and *Xenopus* retinal photoreceptors (46–48). Post-translational activation of TPH has been described in the *Xenopus* retinal photoreceptors, where TPH is believed to be the rate-limiting enzyme in melatonin synthesis (49). Although it is likely that 14-3-3 proteins participate in the regulation of pinealocyte TPH activity, this issue remains to be investigated.

Arylalkylamine N-*acetyltransferase*

AANAT is the penultimate enzyme in melatonin synthesis. Changes in the activity of AANAT control the large changes in melatonin synthesis which occur in all vertebrates; hence the moniker 'the melatonin rhythm enzyme'. AANAT protein, AANAT activity and rate of melatonin synthesis change in parallel. The amount of AANAT protein is regulated to a large degree by controlling the rate at which the enzyme is destroyed by proteasomal proteolysis, which is prevented by a PKA mechanism (50). In many vertebrates, including primates, ungulates and some teleost fish, this proteasomal proteolysis-dependent mechanism is the sole regulatory mechanism, because AANAT mRNA does not change significantly on a day/night basis. It also appears to be the sole mechanism involved in the rapid ($t_{½}$ approximately 3 min) light-induced decrease in vertebrate AANAT protein and activity.

14-3-3 binds to AANAT in a phosphorylation-dependent manner (51, 52), involving PKA phosphorylation of the two PKA sites in AANAT which flank the catalytic core of the enzyme (Fig. 5). Phosphorylation of $_{28}RRHT_{31}$ by PKA is of special importance because it generates a high affinity 14-3-3 binding

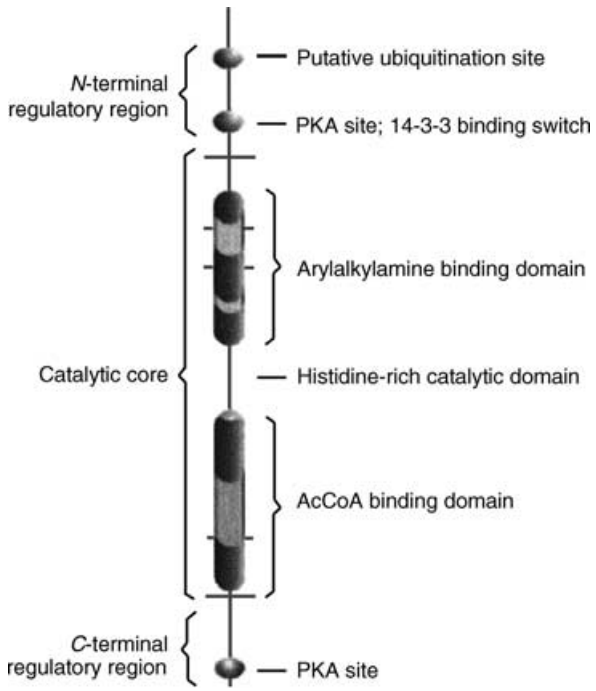

FIG. 5. Schematic structure of arylalkylamine *N*-acetyltransferase (AANAT) illustrating the functional domains of the protein.

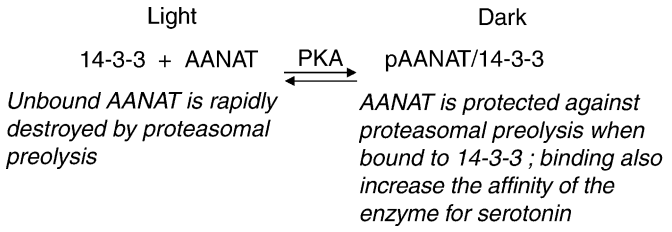

FIG. 6. Control of AANAT activity and stability in the mammalian pinealocyte.

motif: $_{28}$RRHpTLP$_{33}$. Phosphorylation of the *C*-terminal PKA site also occurs (unpublished results, Weller and Klein) and is likely to be important in 14-3-3 binding, as discussed below.

Binding of AANAT to 14-3-3 has several downstream effects on the biology of the enzyme (51, 52). Perhaps most importantly, it has been proposed to prevent destruction of the enzyme by proteasomal proteolysis (53). This alone may be responsible for the large changes in the rate at which AANAT is accumulated in those species in which AANAT mRNA does not change dramatically over 24 h. In the presence of high amounts of mRNA, it is likely that AANAT protein is continually produced and that day/night differences occur because of PKA-mediated phosphorylation of AANAT, which promotes binding to 14-3-3 and prevents destruction. It appears that the AANAT/14-3-3 complex is not entirely stable, and that dissociation occurs continually (i.e. a balance between bound and free AANAT exists). In the absence of cAMP, the balance will shift to the free and unprotected form, leading to proteolysis (Fig. 6).

In addition to the influence of 14-3-3 on the stability and half-life of AANAT, there are two other effects of 14-3-3 binding (51, 52). One is to increase the affinity of the enzyme for serotonin (5-HT), facilitating the conversion of serotonin to *N*-acetylserotonin at night when the concentration of 5-HT is low; a second is to block dephosphorylation of AANAT, which is consistent with phosphate groups of AANAT being directly involved in the 14-3-3/AANAT interaction and, as a result, being inaccessible to phosphatases (51, 52).

The structure of 14-3-3 bound to pAANAT$_{1\text{-}201}$ has been determined (52), representing the first solution of the structure of a complex containing 14-3-3 bound to a fully active protein. This structure was obtained using a truncated form of the enzyme, lacking the *C*-terminal PKA/14-3-3 motif within AANAT$_{202\text{-}207}$. The stoichiometry of the resulting complex is two molecules of pAANAT$_{1\text{-}201}$ bound to one 14-3-3 dimer (Fig. 7).

In the crystal structure of the pAANAT/14-3-3 complex, AANAT molecules contact the central cavity of the 14-3-3 dimer and $_{28}$RRHpTLP$_{33}$ interacts with the 14-3-3 binding groove, as described above. The mode of binding in this region is very similar to that seen in the phosphopeptide structures (1–4, 52). Residues of $_{28}$RRHpTLP$_{33}$ bind in an extended conformation within the groove with numerous hydrogen bonds between pAANAT main-chain and side-chain atoms and residues of 14-3-3. Most notably, the phosphate group of pThr-31 is bound to 14-3-3 through direct hydrogen bonds with Arg-56, Arg-127, and Tyr-128; these bonds appear to be the critical elements explaining how phosphorylation converts AANAT to a high affinity binding partner of 14-3-3.

Analysis of the 14-3-3/pAANAT structure revealed many other contacts between the two binding partners. These are located far removed from the 14-3-3 recognition grooves. Indeed, of the approximately 5000 Å^2 of total surface area that becomes solvent-excluded upon complex formation, only approximately 41% is due to the interaction between the extended *N*-terminal region of pAANAT containing pThr-31 and the 14-3-3 binding groove. Curiously, much of the remaining interaction surface involves a region of AANAT that is highly flexible (52, 54), termed loop 1.

Loop 1 is of special interest because, in one conformation, it completes the arylalkylamine binding pocket; in another, the arylalkylamine binding pocket is incomplete and loop 1 occupies the acetyl CoA binding site. When AANAT is bound to 14-3-3, the first conformation is maintained; this probably explains why 14-3-3 binding lowers the K_m for arylalkylamine.

Therefore, it appears that one of the roles of 14-3-3 may be to modulate the conformation of AANAT into an optimal (or 'pre-assembled') form for substrate binding. It remains an outstanding issue of 14-3-3 biology to determine if this conformational modulation is specific to AANAT or is more generalizable. For example, is this mechanism involved in kinetic changes in TPH and TH which are induced by 14-3-3?

It should be noted that there are differences in the stoichiometry suggested by studies using the truncated form of the enzyme (two molecules of AANAT$_{1\text{-}201}$ bound to one 14-3-3 dimer, as described above) and that suggested by chromatographic studies with the full-length form of the protein, AANAT$_{1\text{-}207}$. These point to a physical complex containing one AANAT$_{1\text{-}207}$ bound to one 14-3-3 dimer. This difference may be linked to the presence of the *C*-terminal PKA site, which could participate in binding of AANAT to 14-3-3. Although this region does not have features of a high-affinity 14-3-3 binding motif, notably the proline residue 2+ relative to the PKA site, it is reasonable to suspect that it might bind to the amphipathic groove of one monomer, reflecting both this interaction and the positioning conferred by binding of the *N*-terminal PKA/14-3-3 site and the numerous other AANAT sites which contact 14-3-3.

Conclusions

14-3-3 proteins are likely to play multiple regulatory roles in pineal photoneuroendocrine transduction, including photodetection, norepinephrine synthesis and release, adrenergic signal transduction and melatonin synthesis; future research may reveal others. Future studies will determine whether 14-3-3 proteins play the hypothetical roles proposed here. This seems likely, which raises the possibility that all 14-3-3 binding partners interact with each other indirectly through competition for common isoforms. It will be important to establish how changes in the abundance of one or another isoform changes function, and what mechanisms regulate intracellular binding through direct post-translational effects on 14-3-3 proteins, including phosphorylation (1–3). Other interesting issues to examine are the determinants of selective isoform binding, whether the abundance of specific isoforms is regulated, what are the most abundant homo- and heterodimers and what regulates dimer formation. It will also be useful to develop pharmacological tools that might selectively alter binding of one target to its 14-3-3 binding partner. It is clear that establishing the role of 14-3-3 proteins in pineal photoneuroendocrine transduction poses an interesting challenge.

The available evidence suggests a broad role for 14-3-3 proteins in pineal photoneuroendocrine transduction as binding partners.

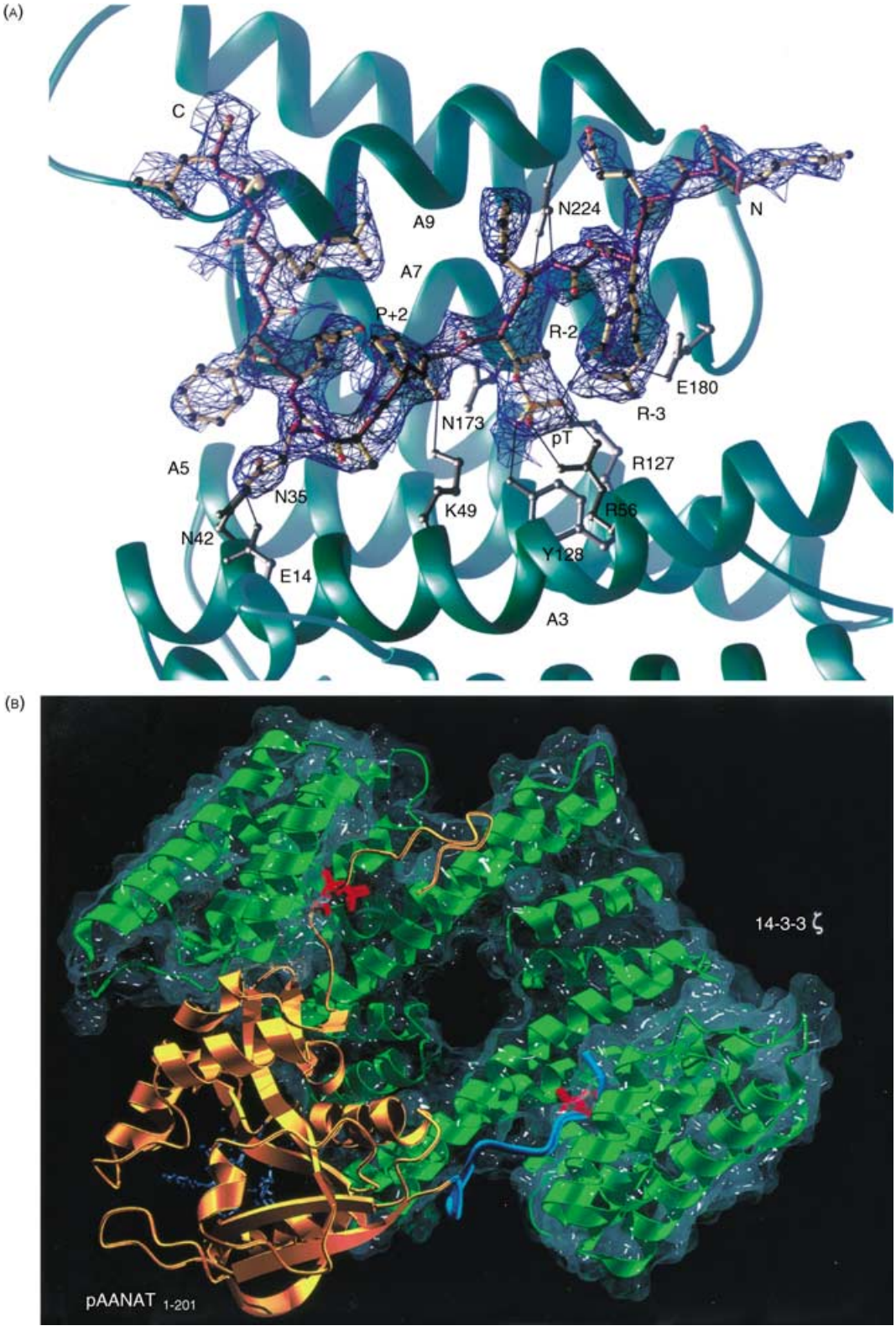

FIG. 7. Structure of the arylalkylamine *N*-acetyltransferase (AANAT)/14-3-3 complex. (A) The electron density of the *N*-terminal 14-3-3 binding motif of $pAANAT_{1\text{-}201}$ within the complex. The antiparallel helices of 14-3-3 are shown in green (labelled A3, A5, A7, A9), and residues Arg-26 to Leu-40 of pAANAT are in red (main chain) and yellow (side chains). (B) Model of the complex between full-length AANAT (residues 18–201 shown in gold, and modelled residues 202–207 in blue) and 14-3-3 (in green). The two phosphothreonine residues are shown in red.

Whereas it is clear they are involved in regulatory processes, it is not known whether regulation is mediated entirely through phosphorylation of a binding partner, or if regulation may also occur through 14-3-3 proteins. If this were the case, the entire transduction process could be controlled through changes in the relative abundance of one or two isoforms.

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